REMARKS

Status of the claims

Claims 1-17, 21-23 are pending in the application. By this amendment, claims 1, 5, 10, 14, 17, and 21 are amended, claims 4, 8, 13 and 23 are cancelled, and new claims 24-41 are added. Thus, following entry of this amendment, claims 1-3, 5-7, 9-12, 14-17 and 21-41 are pending.

The outstanding rejections under Section 112, second paragraph, Section 102, and Section 103 have been withdrawn. A new rejection is levied under Section 112, first paragraph (enablement). Applicants note that claims 1 and 10 have been amended and now recite that the AAV cap gene and the AAV rep gene are stably integrated into the mammalian cell's genome, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell. New claim 25 recites the similar language.

Support for the new and amended claims is found in the specification and previously pending claims, for example, in the specification at page 34, lines 12-14; page 23, lines 19-21; Example 10; and previously pending claims 16 and 17.

With respect to all amendments and cancelled claims, Applicant has not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicant reserves the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional application. Attached hereto is a marked up version of the changes made to the specification by the current amendment with additions underlined and deletions bracketed. The attached page is captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE"**.

Claim Objections

Claims 4 and 13 are objected to under 37 C.F.R. 1.75 (c), as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant submits that it is clear that the term "heterologous promoter" in claims 4 and 13 refer to the "helper virus inducible heterologous promoter" recited in claims 1 and 10, and thus, claims 4 and 13 do further limit claims 1 and 10.[1] However, claims 4 and 13 are canceled, thus mooting this objection. Withdrawal of this objection is respectfully requested

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-17 and 21-23 are rejected under 35 U.S.C. 112, first paragraph, because the specification does not reasonably provide enablement for full scope of the claimed embodiment. The rejection appears to rely on two arguments: (1) that the term "stably integrated" allegedly encompasses an episomally carried plasmid; and (2) the specification "does not provide sufficient guidance for one skilled in the art to make and/or use two heterologous promoters in the claimed invention, wherein the cap gene is operably linked to a distinct heterologous promoter than the promoter operably linked [to] the rep gene", and that "the specification only produces a plasmid comprising AAV rep gene and AAV cap gene operably linked to the same heterologous promoter (Figure 1)." The Office also states that the specification enables stable integration of AAV rep and cap into a mammalian cell line, "wherein the genes are stably integrated into the cell's genome"; and further enables "a plasmid comprising AAV rep gene and AAV cap gene operably linked to the same heterologous promoter." *See* Office Action, page 4. Applicant traverses this rejection.

By this amendment, claims 4, 8, 13 and 23 have been canceled and claims 1 and 10 now recite that the AAV cap gene and the AAV rep gene are stably integrated into the mammalian

[1] Applicant acknowledges that use of "the" rather than "a" could have made the intended reference to the antecedent promoter more clear.

cell's genome, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell. Applicant notes that the Examiner pointed out that the specification states that "stable integration of a polynucleotide into a cell means that the polynucleotide has been introduced into a chromosome or mini-chromosome of the cell and, therefore, becomes a relatively permanent part of the cellular genome". *See* specification at page 23, lines 19-22. Applicant believes that this amendment clarifies that stably integrated, as used in the previously pending claims, means stably integrated into the mammalian cell's genome. Applicant notes that the Examiner stated that the specification enables stable integration of AAV rep and cap into a mammalian cell line, "wherein the genes are stably integrated into the cell's genome." *See* Office Action, page 2-3. Withdrawal of this rejection is respectfully requested.

The Office Action also states that the "specification does not provide sufficient guidance for one skilled in the art to make and/or use two heterologous promoters in the claimed invention, wherein the cap gene is operably linked to a distinct heterologous promoter than the promoter operably linked [to] the rep gene", and that "the specification only produces a plasmid comprising AAV rep gene and AAV cap gene operably linked to the same heterologous promoter (Figure 1)." *See* Office Action, pages 3-4. Applicants respectfully traverse this rejection.

Applicant respectfully submits that the present claims are fully enabled in view of the knowledge of one of ordinary skill in the art, and in view of the specification.

First, Applicant traverses the Office's contention that "the specification only produces a plasmid comprising AAV rep gene and AAV cap gene operably linked to the same heterologous promoter (Figure 1).[2]" *See* Office Action, pages 3-4. *See* Office Action, page 4. By contrast,

[2] Applicant notes that the Office Action also cites "page 27" of the specification for this proposition, but does not identify any portion of page 27 that he relies upon. *See* Office Action, page 4, second full paragraph. Should the Examiner maintain the present rejection, Applicant

the specification (including Figure 1) clearly discloses a mammalian cell which comprises a stably integrated AAV cap gene operably linked to p40 promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter. Specifically, review of Example 1 reveals that the p40 cap promoter[3] is present in the construct disclosed in Figure 1. The construction of plasmid pMt-rep/cap//pKO-neo, as shown in Figure 1, is described in Example 1 at pages 34-35 of the specification. Example 1 states that

> [t]his construction effectively removes both ITR's and substitutes the mMT-1 promoter for the p5 promoter while maintaining all of the AAV reading frames, [and] the p19 and p40 promoters. . . .

Specification, page 34, lines 19-22.

Accordingly, it is evident that the specification does not "*only* produce[] a plasmid comprising AAV rep gene and AAV cap gene operably linked to the same heterologous promoter," as stated by the Examiner in the rejection. Thus, reliance on this ground in the rejection is clearly improper. Reconsideration and withdrawal of this rejection is respectfully requested.

Further, Applicant submits that the Examiner has not made a *prima facie* case of enablement in the present rejection. The law is clear that a specification which teaches how to make and use the invention in terms which correspond in scope to the claims must be taken as satisfying the enablement requirement unless there is reason to doubt the objective truth of the teachings of the specification. *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971). It is incumbent upon the Examiner to explain why one skilled in the art would doubt the truth of statements made in the specification, and provide back up assertions with acceptable and specific evidence. Id. at 370.

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would appreciate it if the Examiner could identify where on page 27 he finds support for this statement.

[3] It is well-known in the art that p40 is the endogenous cap promoter. *See also* specification at *e.g.*, page 4, lines 1-3; page 5, lines 9-13.

By contrast, in the present rejection, the Examiner states

> [t]he specification cites several articles displaying the state of the art for the difficulty of producing a cell for efficiently producing AAV mainly due to the activities of Rep protein (pages 5-16) In view of the state of the art cited by the specification, it is not apparent how one skilled in the art can use a heterologous promoter to control the expression of cap. . . .

Office Action, page 4.

Applicant submits that this cursory discussion, which concerns the difficulty of rep protein expression, has no bearing on any alleged difficulty of expression of cap protein.[4] No evidence is provided that pertains to cap gene expression. Indeed, even the Office Action notes that the "difficulties" discussed in the specification "mainly relate to the activities of Rep protein." *See* Office Action, pages 4-5. Absent evidence to the contrary, the specification must be assumed to be enabling. Because the Examiner has failed to provide acceptable and specific evidence, the specification must be assumed to be enabling. Prompt withdrawal of this rejection is respectfully requested.

Applicant further respectfully submits that the present claims are fully enabled in view of the knowledge of one of ordinary skill in the art, and in view of the specification. For example, the specification teaches (1) how to produce a packaging cell line, including at least the following: selection and design of constructs (*e.g.* at pages 26-30); selection of promoters (*e.g.*, at pages 26-27); testing promoter function and rep and cap gene expression (*e.g.* at page 27); stable integration of rep and cap genes (*e.g.* at pages 27-28); selection of suitable cells and assessment of stable integration (*e.g.*, at pages 28-29); (2) generation of rAAV vectors (pages 30-33); use of cell line for assaying AAV titers (pages 33-34); and (4) 11 working examples (pages 34-48). Applicant note that the specification describes a variety of suitable promoters and

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[4] Applicant notes that page 5-16 of the specification (as referenced by the Examiner) concern the difficulties associated with the expression of the rep protein.

further notes that "a large number of promoters are known in the art . . . " (*see* specification at page 33-34). Accordingly, withdrawal of this rejection is respectfully requested.

CONCLUSION

Applicant believe that the claims are in condition for allowance. Early notification to that effect is earnestly solicited. Should Examiner Whiteman find any issues outstanding after consideration of this Amendment, he is respectfully requested to contact the undersigned at (650) 813-5651.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicant petition for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 226272001403. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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By: Catherine M. Polizzi

Catherine M. Polizzi
Registration No. 40,130

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5651
Facsimile: (650) 494-0792

"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

In the claims

Please amend the claims as follows:

1. (Amended) A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

(a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein the AAV cap gene and the AAV rep gene are stably integrated into the mammalian cell's genome, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell;

(b) replicating the cell of step (a) to produce a population of cells; and

(c) introducing a helper virus to the population of cells of step (b);

(d) wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

5. (Twice amended) The method according to any of claims 1-3[claim 4], wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

10. (Amended) A mammalian cell for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a promoter, and a stably integrated rep gene operably linked to a helper virus-inducible heterologous promoter; wherein the cap gene and the rep gene are stably integrated into the mammalian cell's genome; wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; [and] wherein said cell exhibits helper-virus-inducible expression of said stably

integrated AAV rep gene; and wherein said mammalian cell is prepared by introducing a single plasmid comprising rep and cap arranged as in the AAV genome into the mammalian cell.

14. (Twice amended) The AAV packaging cell of any of claims 10-12[claim 13], wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

17. (Twice amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) introducing a helper virus into an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and

(b) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

21. (Amended) A method of determining the infectious titer of an rAAV vector preparation, comprising the steps of:

(a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

(c) determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.